

Research Article

Titrimetric and Spectrophotometric Methods for the Assay of Ketotifen Using Cerium(IV) and Two Reagents

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Received 22 February 2013; Revised 15 May 2013; Accepted 29 May 2013

Academic Editor: Jan Åke Jönsson

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One titrimetric and two spectrophotometric methods are described for the determination of ketotifen fumarate (KTF) in bulk drug and in tablets using cerium(IV) as the oxidimetric agent. In titrimetry (method A), the drug was treated with a measured excess of cerium(IV) in H_2SO_4 medium and after a standing time of 10 min, the surplus oxidant was determined by back titration with iron(II). The spectrophotometric procedures involve addition of a known excess of cerium(IV) to KTF in acid medium followed by the determination of unreacted oxidant by reacting with either *p*-dimethyl amino benzaldehyde and measuring the resulting colour at 460 nm (method B) or *o*-dianisidine and subsequent measurement of the absorbance of coloured product at 470 nm (method C). Titrimetric assay is based on a 1 : 2 reaction stoichiometry between KTF and cerium(IV) and the method is applicable over 2–18 mg range. In spectrophotometry, regression analysis of Beer's law plots showed a good correlation in 0.4–8.0 and 0.4–10.0 g mL^{-1} KTF ranges for method B and method C, respectively, and the corresponding molar absorptivity coefficients are calculated to be 4.0×10^4 and $3.7 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1}$.

1. Introduction

Ketotifen fumarate (KTF) (Figure 1), chemically known as 4,9-dihydro-4-(1-methyl-4-piperidinylidene)-10H-benzo[4,5]cyclohepta-[1,2-b] thiophen-10-one, which belongs to the group of cyclohepta thiophenones is an antiallergic drug with stabilizing action on mast cells, analogous to that of sodium cromoglycate, and anti- H_1 effect [1]. Ketotifen is given orally as fumarate in the prophylactic management of asthma and is used in the treatment of allergic conditions such as rhinitis and conjunctivitis [2]. Ketotifen is a nonbronchodilator, antiallergic properties, and has a specific anti- H_1 effect [3].

The drug is official in British Pharmacopoeia [4], which describes a potentiometric titration with perchloric acid in acetic anhydride-acetic acid medium. The therapeutic importance of the drug has prompted the development of a variety of techniques for its assay in pharmaceuticals and body fluids. In pharmaceuticals, it is quantified by high performance liquid chromatography [4–10], high performance

thin layer chromatography [11], liquid chromatography with tandem mass spectrometry [12], capillary electrophoresis [13], spectrofluorimetry [14], direct differential pulse polarographic and adsorptive-stripping voltammetry [15], and UV spectrophotometry [16, 17]. Potentiometric titration methods employing polymeric membrane sensors were also developed, where the method involved potentiometric titration of the drug with sodium tetraphenylborate [18, 19]. Determination of KTF has also been carried out based on chemiluminescence [20–22]. Coulometric titration of KTF in pure drug and in tablet forms using biamperometric end-point detection was developed by Ciesilski et al. [23].

To the best of our knowledge, no visual titrimetric method has ever been reported for KTF. Visual titrimetry and visible spectrophotometry may serve as a useful alternatives to many of the aforesaid sophisticated techniques because of their cost-effectiveness, ease of operation, sensitivity, fair accuracy, precision, and wide applicability.

Several visible spectrophotometric methods based on colour reactions involving the amino or thiophene of the

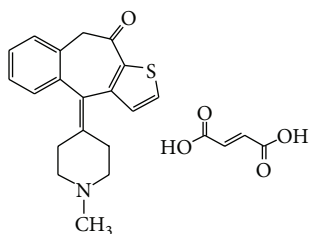


FIGURE 1: Structure of KTF.

ketotifen molecule can be found in the literature. El-Kousy and Bebawy [24] have developed three methods for the assay of KTF in bulk drug and in tablets; the first method was based on extraction of drug-cobalt thiocyanate ternary complex with methylene chloride and estimation by indirect atomic absorption method via the determination of the cobalt content in the complex after extraction in 0.1M hydrochloric acid at 240.7 nm. The second method is based on the formation of orange red ion pair by the reaction of KTF and molybdenum thiocyanate with absorption maxima at 469.5 nm in dichloromethane. The third method was a charge transfer reaction between drug and 2,3-dichloro-5,6-dicyano-p-benzoquinone in acetonitrile medium. Sastry and Naidu [25] have determined the drug in tablets and syrup based on the reduction of Folin-Ciocalteu reagent to a blue coloured chromogen measurable at 720 nm, in the same article; KTF was treated with a measured excess of N-bromosuccinimide (NBS), the unreacted oxidant was reacted with celestine blue, and the change in absorbance was measured at 540 nm. The third method was based on the formation of colored species by the coupling of the diazotized sulphanilamide, and absorbance of the colored species was measured at 520 nm.

There are three reports on the use of ion-pair reaction for the assay of KTF. An extractive spectrophotometric procedure was based ion-pair reaction using Azocarmine G as ion-pair reagent at pH 1.5. The ion-pair complex extracted into CHCl_3 was measured at 540 nm [25]. Sane et al. [26] have described four extractive spectrophotometric procedures based on this reaction using bromophenol blue, bromothymol green, bromothymol blue and bromocresol purple, as ion-pair reagents in acidic medium. The drug has also been determined spectrophotometrically based on ion-pair complex formation with bromocresol green [27] at pH 3.0 followed by extraction into chloroform and measurement at 423 nm. Beer's law is obeyed over the concentration range $5.15\text{--}61.91\ \mu\text{g mL}^{-1}$ KTF. The charge-transfer reaction of KTF with picric acid was developed by Vachek [28]. The method involves extraction of the picric acid-drug complex into chloroform and measurement of the extract at 405 nm.

The reported spectrophotometric methods suffer from some other disadvantage such as narrow linear range, poor sensitivity, dependence on critical experimental variables, tedious and time-consuming extraction steps, heating step, and/or use of expensive reagent or large amounts of organic solvents as indicated in Table 1.

From the foregoing paragraphs, it is clear that cerium(IV) despite its strong oxidizing power, versatility, and high stability in solution has not been applied for the assay of ketotifen. This paper describes for the first time the application of acidic cerium(IV) to the titrimetric and spectrophotometric determination of KTF using *p*-dimethyl amino benzaldehyde and *o*-dianisidine as chromogenic agents. cerium(IV) has earlier been widely applied for the assay of a variety of pharmaceuticals [29–33].

2. Experimental

2.1. Apparatus. A Systronics model 106 digital spectrophotometer (Systronics, Ahmedabad, Gujarat, India) with 1 cm path length matched quartz cells was used to record the absorbance values.

2.2. Materials. All chemicals used were of analytical reagent grade. Double distilled water was used throughout the investigation. Pharmaceutical grade KTF (99.78 percent pure) was procured from Cipla India, Ltd., Mumbai, India, as a gift and used as received. Asthafen-1 (Torrent pharmaceuticals, Sikkim, India) and Ketasma-1 (Sun pharmaceuticals, Sikkim, India) tablets were purchased from local commercial market.

2.3. Reagents and Chemicals. Cerium(IV) Sulphate (0.005 M). An approximately 0.01 M solution was prepared by dissolving about 2.52 g of the chemical $\text{Ce}(\text{SO}_4)_2 \cdot 4\text{H}_2\text{O}$ (Loba Chemie Pvt. Ltd., Mumbai, India) in 0.5 M sulphuric acid with the aid of heat and diluting to 250 mL with the same acid and standardized using standard ferrous ammonium sulphate solution (FAS) [34]. It was used for titrimetric work and diluted to obtain working concentrations of 300 and $100\ \mu\text{g mL}^{-1}$ cerium(IV) for spectrophotometric methods B and C, respectively, with the same solvent.

Perchloric Acid (4 M). Perchloric acid (4 M) was prepared by diluting appropriate volume of commercially available acid (70%; Merck, Mumbai, India) with water.

Sulphuric Acid (5 M). A 5 M sulphuric acid was prepared by appropriate dilution of concentrated acid (98%; Sp. gr. 1.84, Merck, Mumbai, India) with water and used in both methods B and C.

Ferroun Indicator. It was prepared by dissolving 0.742 g of 1,10-phenanthroline monohydrate in 50 mL of 0.025 M ferrous sulphate solution (0.348 g of ferrous sulphate heptahydrate in 50 mL water).

***p*-Dimethylamino Benzaldehyde (*p*-DMAB) (0.5%).** An accurately weighed 0.5 g of *p*-DMAB (Merck, Mumbai, India) was transferred to a 100 mL volumetric flask and dissolved in 4 M HClO_4 and the volume was made up to the mark with the same solvent.

***o*-Dianisidine (ODS) (0.05%).** An accurately weighed 50 mg of *o*-dianisidine (Merck, Mumbai, India) was transferred to

TABLE 1: Comparison of the performance characteristics of the proposed methods with the existing visible spectrophotometric methods.

Serial number	Reagent(s) used	Methodology	λ_{\max} (nm)	Linear range ($\mu\text{g/mL}$) ($\epsilon = \text{L/mol/cm}$)	Remarks	Reference
1	(a) Molybdenum thiocyanate (b) DDQ	Methylene chloride extractable ion-pair complex measured	469.5 588	5–37.5 10–80	Employs unstable oxidant, narrow linear range	[24]
2	(a) F-C reagent (b) NBS-celestine blue (c) Sulphanilamide (d) Azocarmine G	Measurement of the absorbance of blue colored chromogen Unreacted NBS measured using celestine blue in acid medium Measurement of pink colored coupled product of drug with diazotized sulphanilamide with drug Chloroform extractable 1:1 ion-pair complex was measured	720 540 520 540	4–28 2–12 1–10 2.5–25	Multistep, unstable oxidant, strict pH control, and tedious extraction procedure	[25]
3	(a) Bromophenol blue (b) Bromothymol green (c) Bromothymol blue (d) Bromocresol purple	Chloroform extractable 1:1 ion-pair complex was measured	—	0	Required close pH control and narrow linear range and involved tedious time consuming extraction steps	[26]
4	Bromocresol green	Chloroform extractable 1:1 ion-pair complex was measured	423	5.15–61.91	Required close pH control and narrow linear range and involved tedious time consuming extraction steps	[27]
5	Ce(IV)- <i>p</i> -DMAB Ce(IV)- <i>o</i> -dianisidine	Oxidation form of <i>p</i> -DMAB measured Oxidation form of <i>o</i> -dianisidine measured	460 470	0.4–8.0 (4.0×10^4) 0.4–10 (3.7×10^4)	Simple, highly sensitive, extraction free, and use of stable cerium(IV) solution	This work

a 100 mL volumetric flask and dissolved in ethanol and the volume was made up to the mark with the same solvent.

Standard KTF Solution. A stock standard solution equivalent to 2.0 mg mL^{-1} KTF was prepared by dissolving 200 mg of pure drug with water in a 100 mL calibrated flask. This solution was used in the titrimetric work and a working concentration of $20 \mu\text{g mL}^{-1}$ was prepared by stepwise dilution with the same acid and used in method C. For method B, $200 \mu\text{g mL}^{-1}$ KTF solution was prepared separately by dissolving accurately weighed 20 mg of pure drug in 4 M HClO_4 and diluting to volume with the same solvent in a 100 mL standard flask, and the resulting solution ($200 \mu\text{g mL}^{-1}$) was diluted to $20 \mu\text{g mL}^{-1}$ with 4 M HClO_4 and used for assay.

3. Assay Procedures

3.1. Titrimetry (Method A). A 10 mL aliquot of the KTF solution containing 2–18 mg of KTF was transferred into a 100 mL conical flask. To this, 10 mL of 0.005 M cerium(IV) sulphate was added using a pipette, and the contents were mixed well and the flask set aside for 10 min. Finally, the unreacted oxidant was titrated with 0.005 M FAS solution

using one drop of ferroin indicator. Simultaneously, a blank titration was performed, and the amount of the drug in the measured aliquot was calculated from the amount of cerium(IV) reacted.

The amount of KTF in the aliquot was calculated using the formula

$$\text{Amount (mg)} = \frac{\{(B - S) \times M_w \times C\}}{n}, \quad (1)$$

where B = volume of FAS consumed in the blank titration, mL. S = volume of FAS consumed in the sample titration, mL. M_w = relative molecular mass of KTF. C = molar concentration or strength of oxidant in mol L^{-1} . n = number of moles of Ce(IV) reacting with each mole of KTF.

3.2. Spectrophotometry Using *p*-Dimethylaminobenzaldehyde (Method B). Different aliquots (0.2, 0.5, ..., 4 mL) of standard $20 \mu\text{g mL}^{-1}$ KTF solution in 4 M HClO_4 were transferred into a series of 10 mL calibrated flasks by means of a microburette and the total volume in all the flasks was adjusted to 4 mL by adding 4 M HClO_4 . To each flask, 2 mL of 5 M H_2SO_4 and 1 mL of $300 \mu\text{g mL}^{-1}$ Ce(IV) solution were added, and the content was mixed well. After a standing

time of 10 min, 1 mL of 0.5% *p*-DMAB was added to each flask and the volume was made up to mark with 4 M HClO₄. After 5 min, the absorbance of the each coloured product was measured at 460 nm against water.

3.3. Spectrophotometry Using *o*-Dianisidine (Method C). Varying aliquots (0.20, 0.5, . . . , 5.0 mL) of a standard 20 µg mL⁻¹ KTF solution were transferred into a series of 10 mL volumetric flasks using a microburette and the total volume was brought to 5 mL by adding water. To each flask, 1 mL of 5 M H₂SO₄ followed by 1 mL of 100 µg mL⁻¹ Ce(IV) solution were added; and the content was mixed well and kept aside for 10 min at ambient temperature. Finally, 1 mL of 0.05% *o*-dianisidine was added to each flask, the volume was made up to mark with water and mixed well. The absorbance of each solution was measured at 470 nm against water blank after 5 min.

A standard graph was prepared by plotting absorbance against concentration, and the unknown concentration was read from the graph or computed from the regression equation derived using Beer's law data.

3.4. Procedure for the Analysis of Tablet. Two hundred tablets were weighed and finely powdered. An accurately weighed quantity of the tablet powder equivalent to 100 mg KTF was transferred into a 50 mL calibrated flask and about 30 mL water. The content of the flask was shaken for 20 min, and finally the volume was completed to the mark with water. The content was mixed well and filtered through a Whatman No. 42 filter paper. First, 5 mL portion of the filtrate was discarded, and a suitable aliquot of the filtrate (2 mg mL⁻¹ KTF) was then subjected to titrimetric analysis (method A). This tablet extract was diluted stepwise to get 20 µg mL⁻¹ KTF with water, used in spectrophotometry (method C), by following the procedure described earlier. Tablet extract equivalent to 20 µg mL⁻¹ KTF was prepared separately using 4 M HClO₄ and subjected to analysis in spectrophotometric method B by following the assay procedure described earlier.

3.5. Procedure for the Analysis of Placebo Blank and Synthetic Mixture. A placebo blank containing starch (30 mg), acacia (35 mg), hydroxyl cellulose (35 mg), sodium citrate (40 mg), talc (30 mg), magnesium stearate (45 mg), and sodium alginate (35 mg) was prepared by combining all components to form a homogeneous mixture. A 50 mg of the placebo blank was accurately weighed and its solution was prepared as described under "tablets" and then subjected to analysis by following the general procedure.

A synthetic mixture was prepared by adding an accurately weighed 100 mg of KTF to about 150 mg of the placebo mentioned above. The extraction procedure applied for tablets was applied to prepare 2 mg mL⁻¹ KTF solution. This was diluted to get 20 µg mL⁻¹ KTF solutions with respective solvents and used in spectrophotometric methods. Three different volumes of the resulting synthetic mixture solution were subjected to analysis by following the respective general procedure.

4. Results and Discussion

KTF is reported to undergo oxidation with NBS [22], and also cerium(IV) has been used as an oxidimetric reagent for the assay of many oxidisable drugs [29–33]. The present work is based on the oxidation of the sulphur atom of the KTF molecule by a measured excess of cerium(IV) in acid medium and the surplus oxidant was determined by either titrimetry or spectrophotometry.

4.1. Titrimetry. KTF was found to react with Ce(IV) sulphate in sulphuric acid medium. The titrimetric method (method A) involves oxidation of KTF by a known excess of Ce(IV) in sulphuric acid medium with the formation of ketotifen sulphoxide and the unreacted oxidant was determined by back titration with FAS. H₂SO₄ medium was favored over HCl and HClO₄ medium since the reaction was found to yield a regular stoichiometry in the concentration range studied. Reproducible and regular stoichiometry was obtained when 0.65–1.29 M H₂SO₄ concentration was maintained. Hence, 5 mL of 5 M H₂SO₄ solution in a total volume of 25 mL (1M H₂SO₄ overall) was found to be the most suitable concentration for the quantitative reaction between KTF and Ce(IV). Under the optimized reaction condition, there was found to be a definite reaction stoichiometry of 1 : 2 between KTF and Ce(IV) within the range of 2–18 mg of KTF.

4.2. Spectrophotometry. In spectrophotometry (method B), the unreacted Ce(IV) was treated with *p*-DMAB in HClO₄ medium to yield formic acid and *p*-dimethylaminophenol, which upon further oxidation gave the corresponding quinoimine derivative [35], measured at 460 nm, and in method C, the surplus oxidant was treated with *o*-dianisidine in acid medium to form the orange red colour product and measured at 470 nm.

In methods B and C, the increasing concentrations of drug added to a fixed concentration of Ce(IV) yield decreasing concentrations of Ce(IV), and this concomitant decrease in concentration results in decreasing absorbance values of its reaction product with *p*-dimethylaminobenzaldehyde in method B and with *o*-dianisidine in method C.

5. Method Development

5.1. Absorption Spectra. The addition of *p*-DMAB to Ce(IV) resulted in the formation of yellowish red coloured product with maximum absorption at 460 nm against blank, and the decrease in the absorption intensity at 460 nm, caused by the presence of the drug, was directly proportional to the amount of the drug reacted in method B shown in Figure 2.

In method C, the orange coloured product of Ce(IV) with *o*-dianisidine shows maximum absorption at 470 nm against its corresponding reagent blank as shown in Figure 3, and the decrease in the absorption intensity at 470 nm, caused by the presence of the drug, was directly proportional to the amount of the drug.

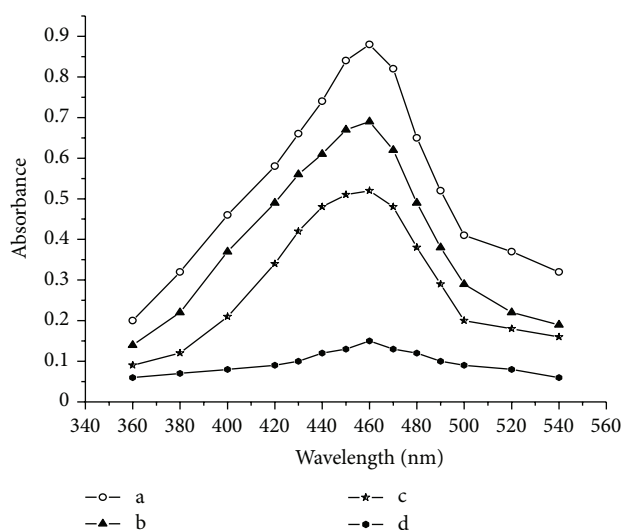


FIGURE 2: Absorption spectra, method B, (a, b, c, and d are absorption spectra of blank, 2, 4, and 8, 20 $\mu\text{g mL}^{-1}$ KTF, resp.).

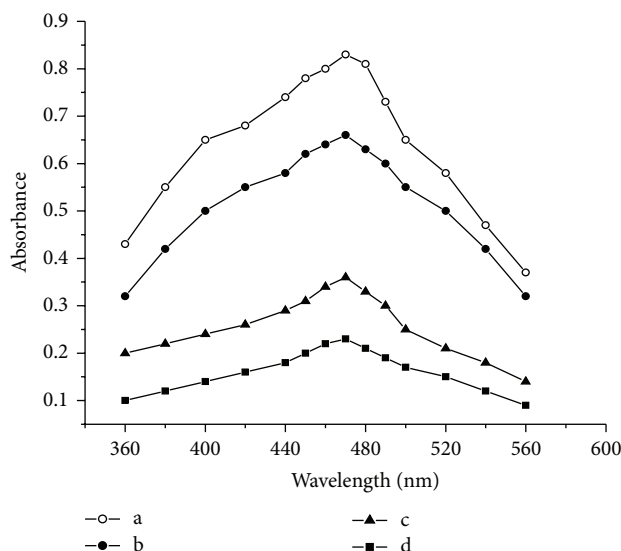


FIGURE 3: Absorption spectra, method C, (a, b, c, and d are absorption spectra of, blank, 2, 6, and 8 $\mu\text{g mL}^{-1}$ KTF, resp.).

5.2. Selection of Reaction Medium. Perchloric acid (4 M) medium was found ideal for rapid and quantitative reaction between KTF and Ce(IV) and to obtain maximum and constant absorbance values due to Ce(IV)-*p*-DMAB reaction product at 460 nm. This may be attributed to the highest oxidation potential of Ce(IV) in HClO_4 ($E_o = 1.75 \text{ V}$) as compared to that of Ce(IV) in H_2SO_4 ($E_o = 1.44 \text{ V}$), HNO_3 ($E_o = 1.61 \text{ V}$), or HCl ($E_o = 1.28 \text{ V}$) [36]. Therefore, all the solutions (ALB, Ce(IV), and *p*-DMAB) were prepared in 4 M perchloric acid throughout the investigation and the same was maintained as reaction medium in method B.

5.3. Optimization of Ce(IV) Concentration for Methods B and C. In method B, to fix the optimum concentration of

Ce(IV), different concentrations of oxidant were reacted with a fixed concentration of *p*-DMAB in HClO_4 medium and the absorbance was measured at 460 nm. A constant and maximum absorbance resulted was $30 \mu\text{g mL}^{-1}$ Ce(IV), and hence different concentrations of KTF were reacted with 1 mL of $300 \mu\text{g mL}^{-1}$ Ce(IV) in HClO_4 medium before determining the residual Ce(IV). This facilitated the optimization of the linear dynamic range over which procedure could be applied for the assay of KTF. In method C, $10 \mu\text{g mL}^{-1}$ Ce(IV) gave a constant and maximum absorbance with *o*-dianisidine at 470 nm, and hence 1 mL of $100 \mu\text{g mL}^{-1}$ Ce(IV) was fixed as optimum.

5.4. Study of Reaction Time and Stability of the Coloured Species. Under the described experimental conditions, the reaction between KTF and Ce(IV) was complete within 10 min at room temperature ($28 \pm 2^\circ\text{C}$). After the addition of *p*-DMAB, a standing time of 5 min was necessary for the formation of coloured product, and thereafter the absorbance of the coloured product was stable for more than an hour in method B.

In method C, with fixed concentrations of KTF, Ce(IV), ODS, and acid, the absorbance was measured by different intervals of time which showed that reaction time is five minutes, and the coloured product is stable for 15 min.

5.5. Effect of Diluent. In order to select proper solvent for dilution, different solvents were tried. The highest absorbance values were obtained when 4 M HClO_4 was used as diluting solvent, and substitution of 4 M HClO_4 by other solvents (methanol, water, 6 M HClO_4) resulted in decrease in the absorbance values in method B. In method C, *o*-dianisidine is prepared in ethanol and drug in water, and water as a diluent gave maximum absorbance.

6. Method Validation

6.1. Linearity and Sensitivity. The present methods were validated for linearity, selectivity, precision, accuracy, robustness, ruggedness, and recovery. Over the range investigated (2–18 mg), a fixed stoichiometry of 1:1 [KTF: Ce(IV)] was obtained in titrimetry which served as the basis for calculations. In spectrophotometry, the calibration graphs were found to be linear from $0.4\text{--}8.0 \mu\text{g mL}^{-1}$ and $0.4\text{--}10.0 \mu\text{g mL}^{-1}$ KTF in method B and method C, respectively, in the inverse manner.

The calibration graphs are given by the equation

$$Y = a + bX, \quad (2)$$

(where, Y = absorbance, a = intercept, b = slope, and X = concentration in $\mu\text{g mL}^{-1}$) obtained by the method of least squares. Sensitivity parameters such as molar absorptivity and Sandell's sensitivity values and the limits of detection (LOD) and quantification (LOQ) were calculated according to the ICH guidelines [37] using the following formulae:

$$\text{LOD} = 3.3 \frac{S}{\text{slope}}, \quad \text{LOQ} = 10 \frac{S}{\text{slope}}, \quad (3)$$

TABLE 2: Sensitivity and regression parameters.

Parameter	Method B	Method C
λ_{\max} , nm	460	470
Colour stability, min	60	15
Linear range, $\mu\text{g mL}^{-1}$	0.4–8.0	0.4–10
Molar absorptivity (ϵ), $\text{L mol}^{-1} \text{cm}^{-1}$	4.0×10^4	3.7×10^4
Sandell sensitivity*, $\mu\text{g cm}^{-2}$	0.0105	0.0136
Limit of detection (LOD), $\mu\text{g mL}^{-1}$	0.39	0.43
Limit of quantification (LOQ), $\mu\text{g mL}^{-1}$	1.19	1.32
Regression equation, Y^{**}		
Intercept (a)	0.8744	0.8082
Slope (b)	−0.0914	−0.0815
Standard deviation of a (S_a)	2.2×10^{-2}	6.6×10^{-2}
Standard deviation of b (S_b)	4.9×10^{-3}	7.3×10^{-3}
Regression coefficient (r)	−0.9998	−0.9990

* Limit of determination as the weight in μg per mL of solution, which corresponds to an absorbance of $A = 0.001$ measured in a cuvette of cross-sectional area 1 cm^2 and $l = 1 \text{ cm}$. ** $Y = a + bX$, where Y is the absorbance, X is concentration in $\mu\text{g mL}^{-1}$, a is intercept, and b is slope.

TABLE 3: Results of intraday and interday accuracy and precision study.

Method	KTF taken $\text{mg}/\mu\text{g mL}^{-1}$	Intraday accuracy and precision ($n = 7$)			Interday accuracy and precision ($n = 7$)		
		KTF found $\text{mg}/\mu\text{g mL}^{-1}$	RE %	RSD %	KTF found $\text{mg}/\mu\text{g mL}^{-1}$	RE %	RSD %
A	5.00	5.06	1.20	1.80	5.12	2.42	2.90
	10.0	10.12	1.62	1.40	10.15	1.51	2.43
	15.0	14.80	1.34	0.94	14.72	1.58	1.92
B	2.0	2.02	1.23	1.72	2.03	1.62	1.18
	4.0	3.96	1.62	0.87	3.94	1.26	1.58
	6.0	6.08	1.33	1.46	6.17	1.90	0.88
C	4.0	3.94	0.73	1.43	3.95	1.14	2.12
	6.0	6.06	1.06	1.09	6.11	1.84	1.79
	8.0	7.90	1.21	1.18	7.88	1.47	1.09

In method A, KTF taken/found, is in mg and it is $\mu\text{g mL}^{-1}$ in method B and C.
RE: relative error (%); RSD: relative standard deviation (%).

where S is the standard deviation of the absorbance of seven blank readings. These are summarized in Table 2.

6.2. Accuracy and Precision. The repeatability of the proposed methods was determined by performing five replicate determinations. The intra-day and inter-day variation in the analysis of KTF was measured at three different levels. The accuracy of an analytical method expresses the closeness between the reference value and the found value. Accuracy was evaluated as percentage relative error between the measured and taken amounts/concentrations. The results of this study are compiled in Table 3 and speak of the excellent intermediate precision ($\% \text{RSD} \leq 2.90$) and accuracy ($\% \text{RE} \leq 2.42$) of the results.

6.3. Robustness and Ruggedness. To evaluate the robustness of the methods, two important experimental variables, namely, standing time and volume of H_2SO_4 , were slightly varied, and the capacity of all the methods was found to remain

unaffected by small deliberate variations. The results of this study are presented in Table 4 and indicate that the proposed methods are robust. Method ruggedness was demonstrated having the analysis done by four analysts and also by a single analyst performing analysis on four different apparatus or instruments in the same laboratory. Intermediate precision values ($\% \text{RSD}$) in both instances were in the range of 0.59%–2.96% indicating acceptable ruggedness. The results are presented in Table 4.

6.4. Selectivity. In the analysis of placebo blank, there was no measurable consumption of Ce(IV) in titrimetry and the same absorbance value as obtained for the reagent blank was recorded in method B and method C, suggesting the noninterference by the inactive ingredients added to prepare the placebo.

In method A, 5 mL of the resulting solution prepared by using synthetic mixture was assayed titrimetrically ($n = 5$) and yielded a percentage of recovery of 102.3 ± 0.62 KTF. In spectrophotometry, 2 mL of $20 \mu\text{g mL}^{-1}$ KTF in method B

TABLE 4: Method robustness and ruggedness expressed as intermediate precision.

Method	KTF taken mg/ $\mu\text{g mL}^{-1}$	Robustness		Ruggedness	
		H ₂ SO ₄ volume, mL (% RSD), $n = 3$	Reaction time, min (% RSD), $n = 3$	Ineranalysis (% RSD), $n = 4$	Intercuvettes/burettes (% RSD), $n = 4$
A	5.0	0.88	1.63	0.81	0.85
	10.0	1.33	1.83	1.93	1.23
	15.0	1.03	0.59	0.89	0.96
B	2.0	2.60	2.11	2.11	2.38
	4.0	2.71	1.36	2.63	1.82
	6.0	1.79	1.80	1.58	2.83
C	4.0	2.66	2.70	2.01	2.71
	6.0	2.45	1.83	2.79	1.99
	8.0	1.72	1.27	1.69	2.96

In titrimetry, standing times were 8, 10, and 12 min.

In spectrometric methods B and C, reaction time, 10 ± 1 min; volume of H₂SO₄ 2 ± 0.2 mL and 1 ± 0.2 mL varied, respectively.

TABLE 5: Results of analysis of tablets by the proposed methods and statistical comparison with the reference method.

Tablets analysed	Label claim, mg/tablet	Reference method	Found* (percent of label claim \pm SD)		
			Proposed methods		
			Method A	Method B	Method C
Asthafen	1	100.8 ± 0.91	101.4 ± 0.89	102.8 ± 1.91	102.1 ± 1.75
			$t = 1.05$	$t = 2.11$	$t = 2.16$
			$F = 1.05$	$F = 4.41$	$F = 3.7$
Ketasma	1	102.3 ± 1.02	100.6 ± 0.98	102.8 ± 1.36	101.3 ± 0.88
			$t = 2.69$	$t = 0.66$	$t = 1.66$
			$F = 1.08$	$F = 1.78$	$F = 1.34$

* Mean value of five determinations;

Tabulated t value at the 95% confidence level is 2.78.

Tabulated F value at the 95% confidence level is 6.39.

and 3 mL of $20 \mu\text{g mL}^{-1}$ KTF in method C when subjected to analysis ($n = 5$) yielded percentage of recoveries of 98.24% and 101.8% KTF with standard deviations of 1.74 and 2.32, respectively. These results complement the findings of the placebo blank analysis with respect to selectivity.

6.5. Application to Tablet Analysis. Commercial KTF tablets were analyzed using the developed methods and also a reference method [38]. The reference method involves potentiometric titration of KTF in anhydrous acetic anhydride—acetic acid medium with acetous perchloric acid. The results obtained were compared statistically by Student's t -test and the variance-ratio F -test. The calculated t and F values did not exceed the tabulated values of 2.78 and 6.39 at the 95 % confidence level and for four degrees of freedom, indicating close similarity between the proposed methods and the reference method with respect to accuracy and precision. These results are summarized in Table 5.

6.6. Recovery Study. To further ascertain the accuracy and reliability of the methods, recovery experiments were performed via standard-addition procedure. Preanalysed tablet

powder was spiked with pure KTF at three different levels and the total was found by the proposed methods. Each determination was repeated three times. The percent recovery of pure KTF added (Table 6) was within the permissible limits indicating the absence of inactive ingredients in the assay.

7. Conclusions

Three methods have been developed for determination of ketotifen fumarate in bulk drug and in its tablets and validated as per the current ICH guidelines. The present visual titrimetric method is simple and economical compared to the reported coulometric method [23]. The spectrophotometric methods are characterized by simplicity since they do not involve any critical experimental variable and are free from tedious and time-consuming extraction steps and use of organic solvents unlike many previous methods as indicated in Table 1. The method using *o*-dianisidine looks more sensitive than most existing methods. Both have additional advantages of ease of operation and possibility of carrying them out with a common laboratory instrument unlike many other instrumental methods reported for ketotifen. They are characterized by high selectivity and comparable

TABLE 6: Results of recovery study via standard addition method with tablet.

Method	Tablet studied	KTF in tablet mg/ $\mu\text{g mL}^{-1}$	Pure KTF added mg/ $\mu\text{g mL}^{-1}$	Total found mg/ $\mu\text{g mL}^{-1}$	Pure KTF* Percent \pm SD
A	Ketasma-1	6.03	3.0	9.24	102.3 \pm 1.98
		6.03	6.0	12.22	101.6 \pm 1.25
		6.03	9.0	15.29	101.3 \pm 2.08
B	Ketasma-1	3.03	1.5	4.54	101.1 \pm 1.26
		3.03	3.0	6.09	102.1 \pm 1.98
		3.03	4.5	7.59	101.4 \pm 1.34
C	Ketasma-1	2.05	1.0	3.06	101.1 \pm 1.63
		2.05	2.0	4.03	99.36 \pm 1.28
		2.05	3.0	5.12	102.4 \pm 1.53

*mg in method A; $\mu\text{g mL}^{-1}$ in methods B and C. *Mean value of three determinations.

sensitivity with respect to the existing methods. The accuracy, reproducibility, simplicity, and cost-effectiveness of the methods suggest their application in the quality control laboratories where the modern and expensive instruments are not available.

Conflict of Interests

The authors declare that they have no conflict of interests with the company name used in the paper.

Acknowledgments

The authors thank Cipla India Ltd., Bangalore, India, for providing pure ketotifen fumarate. The authors are grateful to the authorities of the University of Mysore, Mysore, India, for permission and facilities.

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